



## Characterization and perceptual interaction of key aroma compounds in *Rosa roxburghii* Tratt by sensomics approach

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### ABSTRACT

*Rosa roxburghii* Tratt (RRT) is esteemed for its unique aroma and nutritional value. Analyzing RRT juice samples from four elevations using GC-MS and GC-O, 99 volatile compounds were identified with 37 exhibiting aroma activity. 29 compounds including aldehydes, alcohols, and terpenes, were key contributors to RRT's aroma, based on high OAVs. Aroma recombination and omission tests confirmed 18 key aroma compounds in RRT while 2,5-Dimethyl-4-methoxy-3(2H)-furanone, hexanal, furfuryl acetate, furaoneol acetate, and benzaldehyde as critical aroma compounds. The  $\sigma$ - $\tau$  graph revealed additive interactions among primary aroma compounds, indicating synergistic effects on RRT's overall aroma profile. Longli produced the most fragrant RRT at the lowest elevation of the four producing areas.

### 1. Introduction

*Rosa roxburghii* Tratt (RRT) is a rose plant in the Rosaceae family, a characteristic native wild resource in China. RRT primarily grows in Guizhou, western Hubei, western Hunan, Liangshan, Mianning mountains, and other regions. It is a natural wild fruit with high nutritional value. Currently, a certain scale of artificial cultivation bases of RRT have been established in parts of Guizhou and Henan. RRT is rich in vitamins, tannins, amino acids, and trace elements, with its vitamin C content being particularly notable. It is reported that every 100 g of RRT pulp contains 2075 to 2725 mg of Vc which is 10 times that of kiwi fruits, earning it the title "king of Vc." This ranks it as the top among all fruits and vegetables, gaining public attention and favor, making it a highly potential fruit (He et al., 2016; Zhao et al., 2007). RRT is usually processed into juice, wine, canned products, and dried foods to enter the consumer market because its prickly appearance and sour taste make it unsuitable for fresh consumption.

Another important reason for the popularity of RRT and its products

is its unique aroma, an important indicator to judge the quality of fruits, vegetables, and products. Strong-smelling food is frequently more appealing and has a significant impact on increasing consumer purchasing power. A food's flavor is one of its most important sensory components (Huang, Dong, et al., 2022). Research on the flavor profile of RRT has increasingly focused on the identification and analysis of its volatile compounds. Early studies primarily identified key aroma contributors such as trans-2-hexenol, linalool, and butyl benzoate, and examined changes in these compounds during processes like fermentation (Liang et al., 1992; Lin et al., 2020; Zhou et al., 2015). More recent research has employed advanced techniques such as GC  $\times$  GC-TOF/MS to identify hundreds of volatile compounds and cluster them into aroma-active groups (Ge et al., 2023). Additionally, HS-SPME combined with SAFE has been used to analyze potential aroma-contributing compounds in RRT, the results showed that ethyl 2-methylbutyrate contributed the most to the aroma of RRT (Li, Mo, et al., 2022), while studies have also focused on volatile components from five Guizhou producing areas (Huang, Li, et al., 2022) and potential aroma-active compounds using

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GC-MS and GC-O (Sheng et al., 2023). In addition, Zhou et al. (2015) identified 38 kinds of free volatile components in RRT, with high content of ethyl butyrate, n-hexanol and other substances. However, many of these studies have relied on single extraction methods and have not thoroughly explored the interactions between different volatile compounds. Building on this foundation, comprehensive methodologies have been developed for the extraction, identification, sensory evaluation, and data processing of RRT's flavor. Through the application of multiple extraction techniques, including headspace solid-phase microextraction, liquid-liquid extraction, and solvent-assisted flavor evaporation, 119 volatile compounds have been identified—three to four times the number reported in earlier studies (Li et al., 2021). Among these, 38 compounds have been found to significantly contribute to the overall aroma, with 16 newly identified as key aroma components in RRT juice. Further differentiation of flavor compounds based on altitude has also been achieved, providing deeper insights into how geographic and environmental factors impact the flavor profile of RRT (Li, Tu, et al., 2022).

Despite the fact that the aroma of RRT has been thoroughly studied, systematic research on the main aroma components and how they interact with RRT juice is still absent. The current research rarely combines chemical analysis with sensory evaluation, and it is not clear about the chemical substances of RRT aroma, and there is a lack of research on the mechanism of aroma substances at the molecular level. In each step of the extraction, separation and analysis of flavor substances in food, sensomics approach combines instrumental analysis methods with human perception of flavor, and comprehensively uses methods such as gas chromatography olfactometry (GC-O) combined with odor extract dilution analysis, quantitative measurement, odor activity value, odor recombination and elimination experiments to qualitatively, quantitatively and describe flavor at the molecular level (Steinhaus and Schieberle, 2007). By revealing the chemical essence of food characteristic flavor at the molecular level, it can scientifically study the contribution of aroma components to aroma, and well solve the problems related to the study of RRT aroma mentioned above. Therefore, the aims of this study were (i) to identify the aroma-active compounds of RRT through GC-O and OAVs; (ii) to determine key aroma contributors via aroma recombination and omission experiments, and (iii) to explore the interactions between the key aroma substances of RRT. This research could assist enhance the flavor of RRT juice and its processed products by identifying its main fragrance components.

## 2. Materials and method

### 2.1. Materials and chemicals

Reagents and materials included: cyclohexanone, chromatographic pure, Thermo Fisher Technology (China) Co., LTD.; Series N-Alkanes (C<sub>7</sub>-C<sub>30</sub>), chromatographically pure, Sigma-Aldrich; Anhydrous ethanol, anhydrous sodium sulfate, NaCl, analytically pure, Chengdu Jinshan Chemical Reagent Co., LTD. High purity helium, liquid nitrogen, Guizhou Guorui Gas Technology Co., LTD.

All authentic standards included (Z) -3-hexenol (97 %), 2, 3-butane-diol (98 %), Ethyl isobutyrate (99 %), Isobutyl acetate (> 99.5 %), Ethyl 3-hydroxybutyrate (99 %), Ethyl butyrate (99 %), Ethyl 2-methylbutyrate (99 %), Ethyl hexanoate (> 99 %), (E)-3-hexenal (98 %), Ethyl acetate (98), (E) -3-hexyl acetate (98 %), Ethyl lactate (98 %), Ethyl caprylate (> 99.5), Furaneol acetate (98 %), Furfuryl acetate (99 %), Isoamyl acetate (99 %), Phenylethyl acetate (99 %), Hexanoic acid (99 %), (E) -3-hexenoic acid (98 %), Hexanal (≥99.0 %), (E)-3-Hexenal (98 %), (Z) -3-hexenal (98 %), (E) -2-hexenal (98 %), (E,Z)-2, 4-heptadienal (98 %), benzaldehyde (≥99.0), 2, 5-dimethylbenzaldehyde (98 %), 2, 4-dimethylbenzaldehyde (98 %), 1-pentene-3-one (95 %), 4-s-butoxy-2-butylketone (90 %), 3-pentene Ketone (> 99.5 %), 2, 5-Dimethyl-4-methoxy-3(2H)-furanone (> 97 %), *o*-dimethyl ether (> 99.5), 2-hydroxy-2-methyl-4-heptanone, 3-methyl-2 (5H) -furanone, Naphthalene

(> 99.5 %), Guaiacol (> 99.0 %), Methyl eugenol (98 %) were GC grade and purchased from Shanghai Maclin Reagent Company.

### 2.2. Instruments and equipment

The TQ8040NX Gas Chromatograph Mass Spectrometer (Shimadzu, Japan); OPL275 odorant system (GL Sciences Inc., Japan); AOC-6000 autosampler with a PAL automatic solid-phase microextraction device and a 1 cm-50/30 μm DVB/CAR/PDMS fiber head (CTC, Switzerland); DL-5 M low-speed, large-capacity refrigerated centrifuge (Hunan Pingfan Technology Co.); a bespoke solvent-assisted flavor evaporation device (Glasbläsererei Bahr, Germany); a bespoke Weyl distillation column (Jiangsu San Aisi Scientific Instruments Co. Ltd.); and a heat-gathering thermostatic heating magnetic stirrer (DF-101S) from Gongyi Yuhua Instrument Co. Ltd.

### 2.3. Methods

#### 2.3.1. Preparation of RRT juice

The artificially planted RRT fruits were selected from Longli (LL, 1200 m), Shuicheng (SC, 1600 m), Dafang (DF, 1800 m) and Panzhou (PZ, 2100 m), respectively. The fruits have reached commercial maturity and are fresh without mildew and deterioration. In the ripening stage of RRT in four producing areas, RRT plants with similar age, growth and good growth conditions were selected, and the fresh RRT fruits were evenly picked from all directions of the RRT body. The picking standard was mature RRT fruits with symmetrical fruit, healthy shapes, and no diseases or pests. The sampling time was August 2022. After selecting ripe, fresh RRT fruits that were devoid of mold or rot, the fruit was cleaned, drained, and sliced. The calyx and stems were then cut off to collect the juice. Following a 5-min centrifugation at 4000 rpm and 5 °C, the supernatant of juice was removed and frozen at -20 °C.

#### 2.3.2. Headspace-solid phase microextraction

20 mL headspace vial was filled with a mixture of 8 mL RRT juice and 2 μL cyclohexanone (internal standard), which was then dissolved with 2.88 g of NaCl and sealed with a PTFE spacer. After 15 min of equilibration at 40 °C, 30 min of extraction at 40 °C, and 2 min of splitless injection, the mixture was removed.

#### 2.3.3. GC-MS and GC-O analysis

**2.3.3.1. GC-MS analysis.** The following were the requirements for chromatography: column, a strong polarity 60 m × 0.25 mm InertCap Wax capillary column with 0.25 μm ID; 240 °C is the inlet temperature; the injection volume is 2 μL; split ratio is 5:1; solvent delay time is 3.8 min; ramp-up process is 40 °C for 3 min, then increases to 230 °C at 3 °C/min and holds for 2 min. The carrier gas is He.

The electron bombardment (EI) ion source, electron energy of 70 eV, ion source temperature of 230 °C, mass spectrometry interface temperature of 250 °C, and mass scan range (*m/z*), 29–500 amu were the settings for mass spectrometry.

By using the same chromatographic settings to analyze a range of n-alkanes (C<sub>7</sub>-C<sub>30</sub>), retention indices (RIs) were calculated. Because of this, volatile chemicals could be identified based on having spectra from the National Institute of Standards and Technology mass spectrometry collection (Version 14.0) that matched them by more than 85 %. The volatile compounds were quantified using cyclohexanone as the internal standard. By comparing each compound's relative concentration to the internal standard's area, this information was obtained.

**2.3.3.2. GC-O analysis.** The GC-O analysis was performed using a Shimadzu TQ8040NX gas chromatograph-mass spectrometer with olfactometry capabilities.

The experiment was carried out according to the method reported by

Fan et al. (Fan & Michael, 2006). The samples extracted from RRT were continuously diluted in a 1:1 ratio, and the diluted samples were subjected to GC-O analysis. Each sample was analyzed three times per person to calculate the flavor dilution (FD) values. Where  $FD = 2^n$  ( $n$  is the dilution factor). The final scent intensity rating was determined by taking the average of the three evaluators' scores for each of the nine components that were repeated three times for each individual.

#### 2.3.4. Establishment of an analog matrix of RRT juice

RRT juice matrix was simulated and formulated based on the non-volatile substance content measured in previous studies (Li et al., 2020). Included: fructose 20.11 g, glucose 12.09 g, sucrose 27.57 g, oxalate 0.12 g, tartaric acid 0.05 g, malate 0.28 g, acetic acid 0.17 g, fumaric acid 0.72 g, succinic acid 10.97 g,  $\alpha$ -ketoglutaric acid 10.49 g in 1000 mL deionized water.

#### 2.3.5. Quantitative analysis of aroma-active compounds

Quantification of volatile chemicals from GC-O was done by creating standard curves, for more reasonable results, standard solutions with various gradients of concentration were used to simulate the RRT matrix described in the chapter of 2.3.4.

Six concentration gradients in all were established, 100  $\mu$ L of standard substance (10  $\mu$ L of trans-2-hexene-1-ol, 4-s-butoxy-2-butanol, and Furanol acetate of three standard substances) was added into the matrix solution, and the volume was fixed to 50 mL. The matrix was gradually diluted to obtain the remaining five concentration gradients. At the same time, 2  $\mu$ L of internal standard solution (internal standard was cyclohexanone, 1900  $\mu$ g/L) was added to establish the standard curve of potential aroma active compounds.  $y$  represents the peak area ratio (volatile compound peak area/internal standard peak area). Concentration ratio (concentration of volatile compounds/concentration of internal standard).

#### 2.3.6. Measurement of threshold value

Several volatile substances were found in RRT. Gas chromatography-olfactory technique was used to identify possible odor-active chemicals. The odor activity value of these chemicals was used to calculate their contribution to aroma. Since the same compound may have different threshold values in different substrates, the threshold determination mainly uses water and air as substrates in the existing threshold manuals for aroma compounds. The composition of RRT is complex and rich in multiple sugars and organic acids. To more truly characterize the aroma contribution of volatile compounds of RRT, the standard compounds were placed in simulated RRT matrix for threshold determination.

After the matrix is configured as described in the chapter of 2.3.4, a three-alternative forced-choice(3-AFC) test method is used to determine the threshold according to the China National Institute of Standardization (CNIS) GB/T 22366–2022. The standard substance was added to the matrix solution to configure the test sample with odor stimulation. At the same time, the matrix solution was used to form two reference samples according to the volume of the test sample, and a series of test samples with different concentrations were obtained by dilution of 2 times. Three samples (two reference samples and one test sample) are marked with numbers in the determination. The results of the determination are in two cases, the evaluator chooses the test sample (correct selection) or the reference sample (wrong selection). The olfactory threshold of volatile compounds is obtained by forcing the selection of one sample that is considered different from the other two according to the number only during the assay. The threshold of volatile compounds is calculated by referring to ASTM (1997) documentation on threshold determination(ASM., 1997).

#### 2.3.7. Odor activity value (OAV) calculation

OAVs of the quantified compounds were calculated as the following formula:  $OAV = C_i/OT_i$ , where  $C_i$  was the defined concentration of compound,  $OT_i$  was its odor threshold value measurement in RRT juice

in the chapter of 2.3.4.

#### 2.3.8. Quantitative sensory descriptive analysis of RRT juice

The Guizhou Institute of Technology's fruit wine brewing team provided ten evaluation panel members—five males and five females, ages 22 to 30 and free of smoking history—with prior experience in sensory analysis tasting. The 54-aroma kit (Le Nez du Vin®, France) was used to train the sensory evaluation team for a month, or until they were able to identify every aroma with a 95 % accuracy rate. Before the quantitative sensory descriptive analysis, all panelists were provided with a detailed description of the experiment and had signed a written consent form. Using some of the aroma suites as references for sensory evaluation (chemical standards were substituted if no suitable reference could be found in the aroma suites), the group members discussed the aroma attributes of RRT and chose seven aroma attributes to describe the overall aroma characteristics of RRT. The aroma suites included grassy, woody, honey, caramel, tea-like, pear, and floral aromas. A 6-point intensity scale ranging from 0 to 5 with 1 increment and with 0 = not detectable, 1 = very weak, 3 = moderate, 5 = very strong was used to evaluate the seven sensory qualities, and each sample was examined in triplicate. As indicated in Table S1, various n-butanol concentrations were utilized as a reference for scent strength. The sniffing experiments involved in this study were all done with the knowledge and consent of the participants, and no ethical permission is required in this study. All the smell tests involving the compounds were completed within the safe dose, and the participants did not experience any physical or psychological discomfort after the test.

#### 2.3.9. Aroma recombination

The components were shown in Table S2 (Aroma substances with  $OAV \geq 1$  were selected as the recombinant components. Aroma characteristics of RRT were assessed by sensory staff (5 men and 5 women, 22–30 years old, no history of smoking. The RRT matrix described in 2.3.4 was used, and all 26–29 volatile compounds with  $OAV > 1$  in RRT from four producing areas were added into the matrix according to the content of odor-active compounds obtained from the test results. Meanwhile, the average compound content of one group of four producing areas was added to obtain five groups of RRT recombinant model solutions. The additional amounts are shown in the Table S2. In addition, PZ RRT was selected as the sensory evaluation. Ten trained members of the evaluation team carried out sensory evaluation tests. In this experiment, the team members were required to describe the aroma characteristics of the recombinant model of RRT and PZ RRT rose sample in terms described in 2.3.7 and score and compare with the five-point scale method. Every test was conducted in a sensory evaluation lab at 20 °C, and it was repeated three times for each.

#### 2.3.10. Omission tests

The omission experiment refers to the absence of 29 aroma substances listed in Table S2 (three-point test method is adopted). Each time a compound or a class of compounds is omitted in the experiment, 35 groups of omission experiments are carried out in total. With some adjustments, the findings of this omission experiment are categorized using the methodology outlined by Roessler et al. (1978) as follows: (1) if 7 people answer correctly, it is significant; (2) If 8 to 9 people answer correctly, it is very significant; (3) If 10 people answered correctly, it was extremely significant.

#### 2.3.11. Analysis of the interaction of key aroma components in RRT by $\sigma$ - $\tau$ graph method

The interaction relationship of key aroma components was analyzed by the  $\sigma$ - $\tau$  graph method, which is based on the 2 parameters of [ $\sigma = f(\tau)$ ] for binary mixtures,  $\tau$  refers to the ratio between the aroma intensity of compound A(or compound B) and the sum of the aroma intensity of both, where  $\tau_A = I_A/(I_A + I_B)$  or  $\tau_B = I_B/(I_A + I_B)$ ,  $\sigma$  represents the ratio of the aroma intensity of the mixture to the aroma intensity of the

components (before mixing)  $\sigma = I_{\text{mix}} / (I_A + I_B)$  (Cameleyre et al., 2015; Lytra et al., 2012; Xiao et al., 2018). As the  $\sigma$ - $\tau$  graph shown in Fig. S1, the interaction between aromas is divided into five types. If  $\sigma > 1$ , there is synergy between compounds. When  $\sigma = 1$ , there is complete addition; when  $\sigma < 1$ , there are three cases:  $\max(I_A, I_B) < I_{AB} < I_A + I_B$ , partial addition;  $\min(I_A, I_B) < I_{AB} < \max(I_A, I_B)$ , compromise effect;  $I_{AB} < \min(I_A, I_B)$ , masking effect.

#### 2.4. Statistical analysis

All of the examined data were subjected to a one-way ANOVA using SPSS 21.0, and a  $p < 0.05$  resulted in a significant difference. Data correlation graphs were plotted using chiplot (<https://www.chiplot.online/>), SIMCA 14.1 was used to conduct PCA analysis, and Origin 2019 (OriginLab Corporation, Northampton, MA, USA) was used to create the radar charts. Chemical structures were performed using Indraw (<https://indrawforweb.integle.com/>). Three replicates were analyzed for all experiments. The present study's sensory analysis experiments have adhered strictly to the ethical and professional principles delineated in the principles for Ethical and Professional Practices for the Sensory Analysis of Foods published by the Institute of Food Science & Technology, UK (IFST). And all sensory experiments in this study were carried out at safe doses, and sensory evaluators did not experience physical discomfort after the experiments.

### 3. Results and discussion

#### 3.1. Sensory profiles of RRT juice

Sensory description can convey the characteristics of food to some extent (Vilanova et al., 2011). A detailed aroma profile could demonstrate the diverse importance of volatile components to the overall aroma profile (Feng et al., 2018). In this study, sensory descriptors of RRT juice from four producing areas were constructed through sensory description. The scent profile of RRT juice was described using seven features: "floral," "woody," "pear-like," "honey," "tea-like," and "grassy," as illustrated in Fig. 1. The findings demonstrated that there were variations in the sensory descriptions of RRT in various locations. Among the aroma characteristics of RRT juice, the "grassy" fragrance stands out as the most prominent, which is likely attributed to the presence of aldehydes in RRT (Feng et al., 2018). Additionally, "honey" and "pear" fragrances play important roles in defining the overall sensory profile, followed by "tea-like," "woody," "caramel," and "floral" notes. The PZ sample juice, in particular, exhibits significantly higher

levels of "grassy" and "woody" fragrances compared to samples from other regions, though it has a comparatively lower "caramel" fragrance. On the other hand, the overall sensory properties of the DF sample juice are notably subdued, with lower intensities across most attributes. However, DF and LL samples share similar scores in the "woody" fragrance attribute, while all four regions show comparable scores in the "pear" fragrance attribute. These variations highlight the distinct aromatic profiles across different regions (Staroscik and Wilson, 1982), reflecting the influence of geographical factors on the sensory characteristics of RRT juice.

#### 3.2. Analysis of volatile compounds in the four RRT juice samples

In total, 99 volatile compounds were identified across the four producing regions, of which 61 were common to all samples. These compounds included 20 esters, 6 alcohols, 3 acids, 9 aldehydes, 5 ketones, 2 terpenes, 4 aromatic compounds, and 2 furans. The aldehydes exhibited relatively high content across the sample groups which is in line with previous research (Sheng et al., 2023) ranging from 24.92 % to 68.87 %, while esters were present at relatively lower levels, ranging from 12.04 % to 46.00 %. Among the four regions, the juice from LL exhibited the highest total content of volatile components (108,690.68  $\mu\text{g}\cdot\text{L}^{-1}$ ), while the DF sample had the lowest total content (38,478.99  $\mu\text{g}\cdot\text{L}^{-1}$ ).

Among the identified esters, 44 were present, with 22 being ethyl esters, known for their good volatility and low odor threshold, contributing significantly to the overall fragrance of RRT. Alcohols, another important class of volatiles, varied widely among the samples, with proportions ranging from 7.77 % to 47.81 %. (E)-3-hexenol, a known C6 compound produced when plants are mechanically damaged, was detected in samples from all regions except PZ.

Each region displayed distinct volatile profiles. The LL sample exhibited the highest total content of volatile compounds (108,690.68  $\mu\text{g}\cdot\text{L}^{-1}$ ), characterized by the highest concentrations of esters (46.00 %), terpenes (0.12 %), and aromatics. In contrast, the PZ sample was notable for its elevated levels of acids, aldehydes (53.75 %), ketones, and furans, although it had the lowest proportion of terpenes (0.07 %). The SC sample was distinguished by its high alcohol content, which accounted for 47.81 % of its volatile profile, but it had the lowest content of aldehydes (24.92 %) and furans. Meanwhile, the DF sample exhibited the lowest overall concentration of volatile compounds (38,478.99  $\mu\text{g}\cdot\text{L}^{-1}$ ), reflecting a more subdued aromatic profile compared to the other regions.

#### 3.3. Potential aroma-active compounds identified by GC-O analysis in RRT juice

Aroma extract dilution analysis (AEDA) is based on continuously diluting and smelling the sample aroma extract which can be applied to further elucidate the importance of volatile components and the aroma dilution factor (FD) is used to represent the importance of aroma compounds' contribution to the sample aroma (Feng et al., 2018; Grosch, 1994). The larger the FD value is, the more dilution the aroma substance can still be smelled, that is, the more important the aroma substance is to the aroma of the sample. AEDA method can accurately reflect the aroma contribution of aroma compounds.

There were 20 compounds with the value of  $\text{FD} \geq 2$  in RRT juice from four regions listed in Table S3. Combined with previous studies (Sheng et al., 2023), the majority of the fragrance compounds in RRT juice with higher OSME values also have higher FD values, indicating their significant impact to the juice's aroma. The compound with the highest FD value was 2, 5-dimethyl-4-methoxy-3 (2H) -furanone in the juice from four regions. The other compounds with high FD value in DF sample were ethyl 2-methylbutyrate (128), furaneol (64), (Z) -3-hexenal (64) and ethyl caprylate (16). The other compounds with high value of F in LL sample were ethyl 2-methylbutyrate (256), furaneol (128), ethyl caprylate (64), (Z) -3-hexenal (64), Ethyl butyrate (32), (Z) -3-hexenol (16)

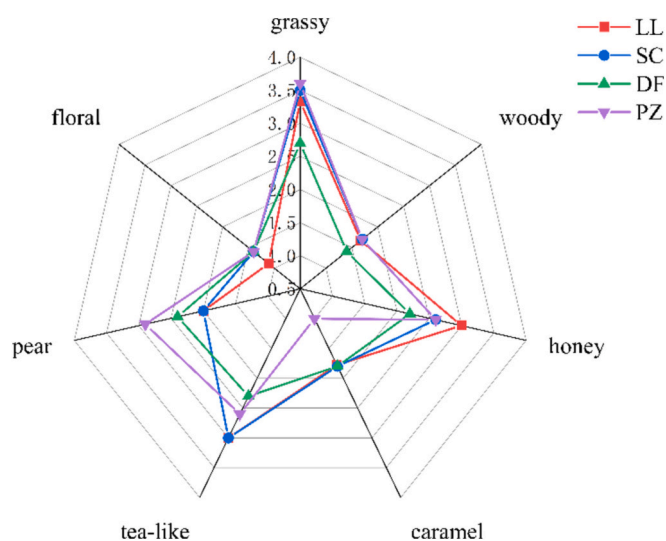


Fig. 1. Quantitative descriptive analysis of RRT juice.

and ethyl 3-hydroxybutyrate (16). The other compounds with high FD values in PZ sample were ethyl 2-methylbutyrate (128), furaneol (128), (Z) -3-hexenal (64), ethyl caprylate (32), 2-butanediol (16) and Ethyl butyrate. The remaining chemicals in RRT juice that had high FD values were 2-methylbutyrate (128), furaneol (64), (Z) -3-hexenal (32), ethyl caprylate (32) and 2-butanediol (16).

### 3.4. Aroma-active substances found in RRT juice according to OAVs analysis

A volatile compound's aroma activity value was computed to find out how much of an aroma it contributed to RRT juice. The ratio of the volatile component content in RRT juice to its odor threshold value was the aroma activity value measured. It was thought to contribute to the aroma of RRT juice when  $OAV > 1$  (Grimm and Steinhaus, 2019). The results were shown in Table 1. Among 37 quantitative compounds, 29 had OAV values  $> 1$ , and Hexanoic acid was the highest in the four regions. Hexanoic acid usually had a similar aroma to "citrus, lemon, and fat" (Steinhaus and Schieberle, 2007; Kim et al., 2020); The outcome showed that the chemical significantly influenced RRT's aroma. Among them, there were 8 compounds with  $OAV > 1000$  in the four regions, as follows: Ethyl butyrate, hexanal, 2, 4-dimethylbenzaldehyde, hexanoic acid, furaneol acetate, benzaldehyde, (E) -3-hexenal, ethyl caprylate, indicating that these substances significantly contribute to RRT's aroma and the content differences of these compounds may also be different due to harvest time (Li, Mo, et al., 2022; Dou et al., 2020). There were 29 compounds with  $OAV \geq 1$  in the juice of RRT. These compounds contributed to the aroma of RRT. Among them, there were 13 compounds with  $OAV > 1000$ , which were Ethyl butyrate, Hexanal, Isoamyl acetate, furfuryl acetate, 2, 4-dimethylbenzaldehyde, hexanoic acid, furaneol acetate, benzaldehyde, Nonanal, ethyl 3-hexenoate, ethyl hexanoate, (E) -3-hexenyl aldehyde, ethyl caprylate. There are three compounds with  $100 < OAV < 1000$ , which are 3-pentanone, 2-methylbutyrate ethyl ester, and ethyl lactate. There were 29 compounds with  $OAV \geq 1$  in the juice of RRT from SC, which contributed to RRT's aroma. There were 10 compounds with  $OAV > 1000$ , which were 3-pentanone, ethyl butyrate, hexanal, furfuryl acetate, 2, 4-dimethylbenzaldehyde, hexanoic acid, Furaneol acetate, benzaldehyde, (E) -3-hexenal, ethyl caprylate. There are five compounds with  $100 < OAV < 1000$ , which are: ethyl 2-methylbutyrate, Isoamyl acetate, Nonanal, ethyl 3-hexenoate and Ethyl hexanoate. There were 29 compounds with  $OAV \geq 1$  in the juice of RRT, which contributed to the aroma of PZ sample. There were 12 compounds with  $OAV > 1000$ , which were ethyl butyrate, hexanal, (E) -2-hexenal, 2, 4-dimethyl benzaldehyde, hexanoic acid, Furaneol acetate, benzaldehyde, nonanal, ethyl 3-hexenal, Ethyl hexanoate, (E) -3-hexenal, ethyl caprylate. Three compounds with  $100 < OAV < 1000$  were 3-pentanone, ethyl lactate, and furfuryl acetate. There were 29 compounds with  $OAV \geq 1$  in the juice of PZ RRT, which contributed to the aroma of PZ RRT, among which 11 compounds with  $OAV > 1000$  including ethyl butyrate, hexanal, furfuryl acetate, 2, 4-dimethylbenzaldehyde, hexanoic acid, furaneol acetate, benzaldehyde, nonanal, ethyl hexanoate, (E) -3-hexenal, ethyl caprylate.

There are four compounds with  $100 < OAV < 1000$  including ethyl 2-methylbutyrate, isoamyl acetate, (E) -2-hexenal, ethyl 3-hexenal. In conclusion, the odor-active compounds of RRT juice from four producing areas mainly comprised of esters, alcohols, aldehydes, and ketones.

### 3.5. Aroma recombination

The overall aroma of the recombinant system is shown in Fig. 2, like the authentic RRT, is mainly characterized by "grassy", "floral" fragrance and "honey" fragrance. By comparing and analyzing the aroma attributes of the real system and the simulated system, it can be found that the aroma profiles of the real system and the recombinant system are very similar but slightly different. This may be because the matrix itself of the recombinant system is very different from that of the

real system, and the establishment time of the system is short, the interaction between compounds is not carried out, or the simulated matrix of the recombinant system does not contain other influencing factors such as non-volatile components interacting with aroma properties.

Due to certain differences in composition types and contents of odor-active substances in different producing areas, corresponding recombinant models were constructed for the four producing areas, and a set of mean recombinant models were added according to the average content of odor-active compounds in the four producing areas for comparison with real RRT samples from the four producing areas. The seven aroma characteristics were scored and compared according to the chapter of 2.3.7. The result is shown in Fig. 2. Compared with the original samples of LL recombination model, the aroma characteristics of "pear", "floral" and "woody" are very similar. The aroma characteristics of "caramel" and "honey" have certain differences but still retain high similarity, while the aroma characteristics of "tea-like" show a quite difference. The intensity of "tea" in the recombination model is very low compared with the real sample. Compared with the real samples, the aroma characteristics of "woody" and "pear" in SC reconstructed model were almost identical. The intensity of "tea-like", "caramel", "honey" and "grassy" in the reconstructed model was lower than that of the real samples, while the intensity of "floral" was higher. In DF recombination model, the aroma characteristics of "tea-like" and "honey" were like those of real samples, while "caramel" was slightly more intense than the real samples, the difference was not very noticeable. The intensity of "grass", "woody" and "floral" were all higher than that of real samples, and the intensity of "pear" was lower than that of real samples. The aromas of "grass" and "woody" in PZ recombination model were identical with those of real samples, while the aromas of "caramel" and "floral" were lower than those of real samples, while the aromas of "caramel" were significantly higher than those of real samples.

The mean recombination model and the real RRT samples from four regions showed certain similarities in aroma characteristics, especially the "woody aroma" and "tea aroma", but the "grass aroma" and "honey aroma" showed higher intensity than the real RRT samples. Since the grassy flavor of RRT may mainly come from Hexanal (Sheng et al., 2023), it is speculated that some substrates are masking the aroma release of Hexanal (the main source of grassy fragrance) in real samples of RRT, and matrix simulation in the recombinant model of RRT could not fully recover the real RRT matrix. This leads to differences in the intensity of aroma characteristics.

Although the aroma attribute intensity of the real RRT sample system and the recombinant model were slightly different, the statistical study's conclusions showed that there was no significant difference between the genuine system and the recombinant system in the seven attributes examination, that is, the simulation system of RRT was successfully constructed. This result also reaffirmed the high degree of accuracy of the earlier study and the main system of aroma identification research methods that were established in this study, including the extraction and extraction technology of aroma substances, the combination of smell screening and aroma extract dilution analysis, quantitative technology based on complementary aroma active compounds, and the computation of OAV value.

### 3.6. Omission tests

The Omission tests of 29 aroma active compounds with  $OAV \geq 1$  were conducted to establish 35 omission models. As is shown in Table 2, 23 omission models and recombinant models were significant, among which 6 omission models were very highly significant, 6 omission models were highly significant, and 11 omission models were significant. As shown in Fig. 3, the results indicated that 18 compounds were key aroma substances in RRTs. The very highly significant models include the omission of furaneol acetate, furfuryl acetate, hexanal, 2,5-Dimethyl-4-methoxy-3(2H)-furanone, hexanoic acid, and the omission

**Table 1**  
Comparison of OAV values of aroma-active compounds in four RRT juices.

NO.	Compound	Standard curve	R <sup>2</sup>	Concentration( $\mu\text{g}\cdot\text{L}^{-1}$ ) <sup>a</sup>			OAV				
				LL	SC	DF	PZ	LL	SC	DF	PZ
1	3-Pentanone	$y = 0.190304x + 0.2472$	0.9978	379.97 ± 17.95 <sup>b</sup>	787.49 ± 30.68 <sup>a</sup>	197.30 ± 4.11 <sup>c</sup>	–	603.13	1249.99	313.17	–
2	Isobutyl acetate	$y = 0.319565x + 0.62912$	0.9987	360.86 ± 11.51 <sup>b</sup>	434.32 ± 13.27 <sup>a</sup>	97.24 ± 2.06 <sup>d</sup>	270.65 ± 89.47 <sup>c</sup>	13.36	16.08	3.60	10.02
3	Ethyl butyrate	$y = 0.699260x - 0.7892$	0.9974	790.62 ± 25.09 <sup>a</sup>	154.10 ± 5.34 <sup>b</sup>	63.77 ± 1.92 <sup>c</sup>	781.76 ± 49.93 <sup>a</sup>	60,817.18	11,853.61	4905.35	60,135.19
4	Ethyl 2-methylbutyrate	$y = 0.668552x + 0.52101$	0.9972	453.56 ± 13.60 <sup>a</sup>	196.80 ± 6.48 <sup>c</sup>	41.95 ± 0.55 <sup>d</sup>	335.53 ± 20.32 <sup>b</sup>	944.92	410.00	87.40	699.03
5	Hexanal	$y = 0.260380x + 0.02391$	0.9945	6738.73 ± 106.39 <sup>a</sup>	2214.02 ± 67.49 <sup>c</sup>	4787.23 ± 161.70 <sup>b</sup>	6736.12 ± 205.21 <sup>a</sup>	37,437.41	12,300.08	26,595.72	37,422.87
6	Isoamyl acetate	$y = 0.176559x - 0.419810$	0.9989	267.11 ± 7.96 <sup>a</sup>	128.53 ± 3.10 <sup>b</sup>	–	–	1780.75	856.89	–	–
7	(E)-2-Hexenal	$y = 0.618,318x - 0.138921$	0.9981	1021.61 ± 44.20 <sup>c</sup>	654.47 ± 28.27 <sup>d</sup>	20,775.84 ± 562.80 <sup>a</sup>	5983.96 ± 6445.41 <sup>b</sup>	89.55	57.37	1821.05	524.51
8	Ethyl lactate	$y = 0.45006x - 0.228192$	0.9978	3146.43 ± 131.79 <sup>a</sup>	220.15 ± 9.06 <sup>c</sup>	1403.35 ± 60.73 <sup>b</sup>	–	229.05	16.03	102.16	–
9	4-s-Butoxy-2-butanone	$y = 0.190,066x + 0.42984$	0.9919	107.65 ± 4.66 <sup>a</sup>	73.24 ± 5.69 <sup>b</sup>	67.60 ± 1.18 <sup>c</sup>	65.14 ± 5.76 <sup>c</sup>	6.42	4.37	4.03	3.88
10	Ethyl 3-hydroxybutyrate	$y = 0.78,567x - 0.43992$	0.9942	61.25 ± 4.08 <sup>a</sup>	–	3.28 ± 1.15 <sup>d</sup>	48.03 ± 8.08 <sup>b</sup>	30.78	–	1.65	24.14
11	Furfuryl Acetate	$y = 0.229729x - 0.529821$	0.9977	228.78 ± 1.38 <sup>b</sup>	105.21 ± 0.77 <sup>c</sup>	35.36 ± 2.76 <sup>d</sup>	364.98 ± 12.41 <sup>a</sup>	3574.65	1643.90	552.44	5702.86
12	2,5-Dimethyl-4-methoxy-3(2H)-furanone	$y = 0.81924x - 0.42192$	0.9946	1615.49 ± 92.24 <sup>b</sup>	1104.52 ± 70.30 <sup>c</sup>	1013.24 ± 45.99 <sup>c</sup>	1948.14 ± 55.33 <sup>a</sup>	3.34	2.28	2.09	4.02
13	Veratrole	$y = 0.248,759x - 0.062812$	0.9952	620.77 ± 18.19 <sup>a</sup>	496.88 ± 8.32 <sup>b</sup>	65.61 ± 4.82 <sup>c</sup>	41.64 ± 1.90 <sup>d</sup>	19.32	15.46	2.04	1.30
14	2,4-Dimethylbenzaldehyde	$y = 0.92363x - 0.100,334$	0.9982	107.41 ± 4.42 <sup>a</sup>	62.70 ± 2.38 <sup>b</sup>	40.78 ± 3.64 <sup>c</sup>	20.62 ± 3.34 <sup>d</sup>	11,073.55	6464.38	4203.78	2125.89
15	2,5-Dimethylbenzaldehyde	$y = 0.52909x + 0.16567$	0.9978	187.39 ± 7.71 <sup>a</sup>	–	71.06 ± 6.36 <sup>c</sup>	35.87 ± 5.84 <sup>d</sup>	48.05	–	18.22	9.20
16	Hexanoic acid	$y = 0.106334x + 0.766913$	0.9986	279.44 ± 13.02 <sup>a</sup>	293.05 ± 19.83 <sup>a</sup>	61.01 ± 4.11 <sup>c</sup>	131.16 ± 9.57 <sup>b</sup>	10,831,075.92	11,358,552.74	2,364,564.64	5,083,803.01
17	Guaiacol	$y = 0.164823x - 0.41621$	0.9987	74.49 ± 2.91 <sup>a</sup>	69.37 ± 3.60 <sup>ab</sup>	45.93 ± 4.12 <sup>b</sup>	15.08 ± 2.29 <sup>c</sup>	42.57	39.64	26.25	8.61
18	Furaneol acetate	$y = 0.14561x - 0.274932$	0.9931	479.22 ± 31.13 <sup>b</sup>	38.50 ± 4.04 <sup>c</sup>	37.24 ± 3.57 <sup>c</sup>	549.03 ± 43.26 <sup>a</sup>	638,954.47	51,334.50	49,648.99	732,042.44
19	Methyl eugenol	$y = 0.251,329x - 0.61421$	0.9956	519.07 ± 11.62 <sup>a</sup>	203.62 ± 4.64 <sup>c</sup>	60.40 ± 1.01 <sup>d</sup>	268.27 ± 4.96 <sup>b</sup>	25.05	9.83	2.92	12.95
20	Benzaldehyde	$y = 0.15642x + 0.26221$	0.9957	74.00 ± 0.89 <sup>a</sup>	35.92 ± 3.76 <sup>c</sup>	34.74 ± 3.32 <sup>c</sup>	52.88 ± 10.98 <sup>b</sup>	2466.52	1197.38	1158.16	1762.81
21	2-Phenylethyl Acetate	$y = 0.669,870x - 0.187321$	0.9942	63.71 ± 2.95 <sup>b</sup>	75.02 ± 2.51 <sup>ab</sup>	40.09 ± 2.30 <sup>c</sup>	84.02 ± 8.43 <sup>a</sup>	3.34	3.93	2.10	4.40
22	Ethyl isobutyrate	$y = 0.660865x - 0.23257$	0.9925	51.65 ± 4.35 <sup>b</sup>	26.45 ± 6.49 <sup>d</sup>	42.88 ± 0.56 <sup>c</sup>	64.86 ± 21.34 <sup>a</sup>	15.30	7.84	12.70	19.21
23	Nonanal	$y = 0.222618x - 0.09184$	0.9955	102.08 ± 1.20 <sup>b</sup>	22.25 ± 0.75 <sup>c</sup>	111.80 ± 7.66 <sup>b</sup>	217.77 ± 59.95 <sup>a</sup>	1701.27	370.79	1863.33	3629.43
24	Ethyl (E)-3-hexenoate	$y = 0.565850x - 0.733191$	0.9937	489.14 ± 13.35 <sup>a</sup>	–	–	140.47 ± 11.13 <sup>b??</sup>	1881.32	–	–	540.28
25	Ethyl hexanoate	$y = 0.61707x + 0.206715$	0.9934	1570.68 ± 31.24 <sup>a</sup>	9.44 ± 0.78 <sup>d</sup>	227.14 ± 5.66 <sup>c</sup>	461.80 ± 280.63 <sup>b</sup>	98,167.44	589.79	14,196.40	28,862.50
26	(E)-3-Hexenal	$y = 0.132997x - 0.247321$	0.9976	3864.12 ± 208.65 <sup>b</sup>	2511.18 ± 139.74 <sup>c</sup>	3661.50 ± 231.74 <sup>b</sup>	5165.64 ± 700.16 <sup>a</sup>	5442.42	3536.88	5157.04	7275.55
27	(E)-3-hexenol	$y = 0.679352x - 0.49828$	0.9918	47.57 ± 1.14 <sup>b</sup>	162.18 ± 5.68 <sup>a</sup>	27.95 ± 7.68 <sup>c</sup>	–	3.71	12.66	2.18	–
28	2,3-Butanediol	$y = 0.105139x - 0.467239$	0.9935	3544.75 ± 343.74 <sup>c</sup>	4781.37 ± 441.03 <sup>b</sup>	1232.93 ± 129.94 <sup>d</sup>	6364.64 ± 51.14 <sup>a</sup>	42.29	57.04	14.71	75.93
29	Ethyl octanoate	$y = 0.188207x - 0.626713$	0.9922	439.57 ± 29.81 <sup>a</sup>	76.90 ± 6.64 <sup>d</sup>	93.21 ± 3.59 <sup>c</sup>	224.43 ± 10.51 <sup>b</sup>	44,401.03	7767.24	9414.96	22,669.22

Note: <sup>a</sup>, different letters represent significant differences; “–” Indicates that the compound was not detected.

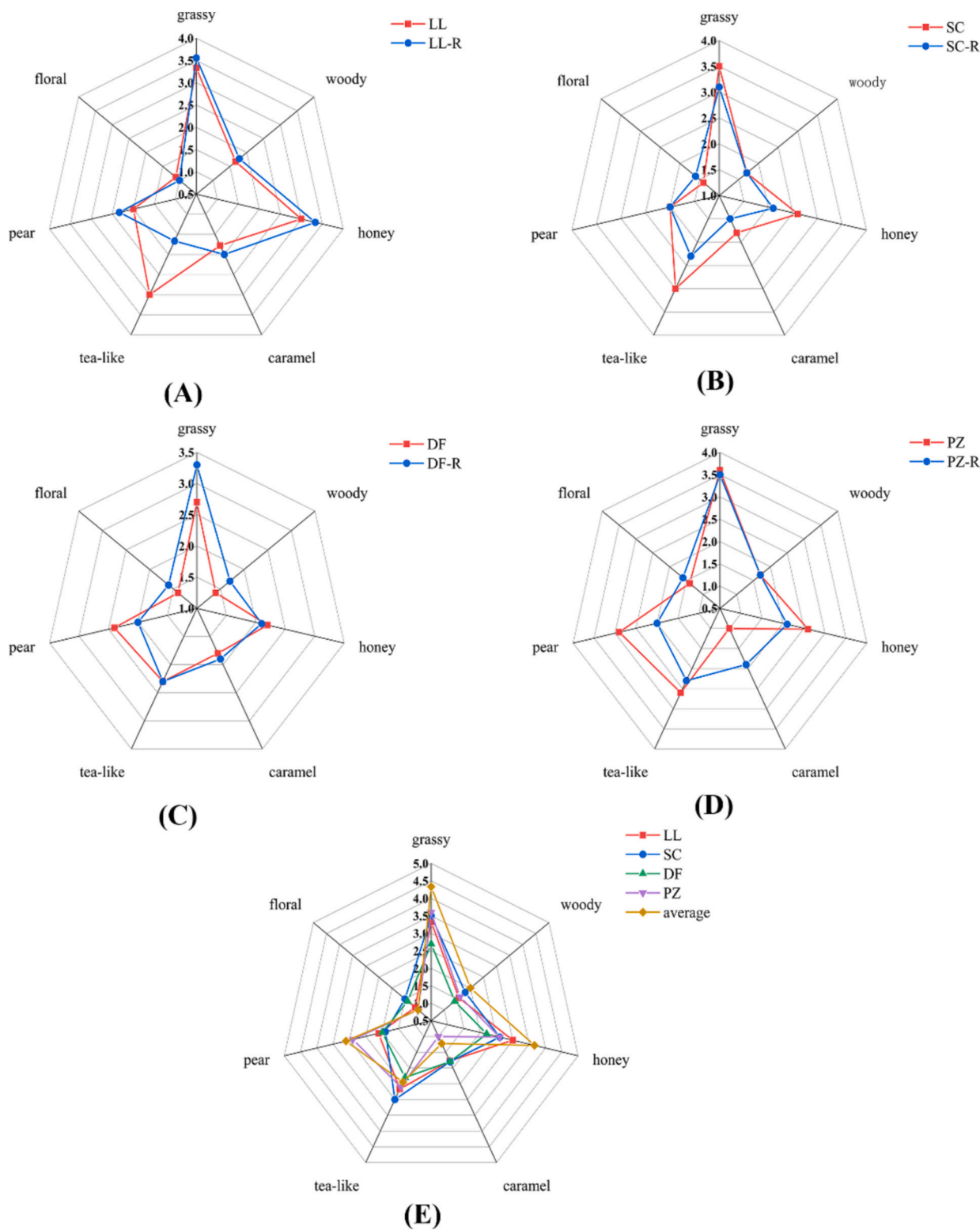


Fig. 2. Comparison of real samples of RRT with recombinant aroma model (radar chart). (A-LL ; B-SC ; C-DF ; D-PZ ; E- Mean recombination model)

of all aromatic compounds. Highly significant models include the omission of ethyl 2-methylbutyrate, ethyl hexanoate, ethyl butyrate, and all aldehydes, (E)-3-hexenal, and (E)-2-hexenal. Significant models included the omission of (E)-3-hexenol, isobutyl acetate, isoamyl acetate, ethyl 3-hydroxybutyrate, nonanal, benzaldehyde, 4-s-Butoxy-2-butanone, Methyl eugenol, and 3-pentenone, all alcohols, all ketones,

all esters. Esters are a crucial class of substances in RRT, and studies have shown that these esters mostly come from RRTs themselves, in addition, the esterification reaction of branched-chain esters during storage and the hydrolysis reaction of high-concentration linear esters will also generate some esters (Liu et al., 2016; Sheng et al., 2023).

**Table 2**  
Omission test of volatiles in RRT.

No.	compounds left out of the complete recombine	n <sup>a</sup>	significance <sup>b</sup>
1	<b>All alcohols</b>	7	*
1-1	2,3-Butanediol	3	
1-2	(E)-3-hexenol	7	*
2	<b>All esters</b>	7	*
2-1	Ethyl isobutyrate	5	
2-2	Isobutyl acetate	7	*
2-3	Ethyl butyrate	8	**
2-4	Ethyl 2-methylbutyrate	9	**
2-5	Ethyl hexanoate	9	**
2-6	Ethyl (E)-3-hexenoate	4	
2-7	Ethyl lactate	5	
2-8	Ethyl octanoate	3	
2-9	Furaneol acetate	10	***
2-10	Furfuryl Acetate	10	***
2-11	Isoamyl acetate	7	*
2-12	2-Phenylethyl Acetate	5	
2-13	Ethyl 3-hydroxybutyrate	7	*
3	<b>All aldehydes</b>	9	**
3-1	Nonanal	7	*
3-2	Hexanal	10	***
3-3	(E)-3-Hexenal	8	**
3-4	(E)-2-Hexenal	8	**
3-5	Benzaldehyde	7	*
4	<b>All ketones</b>	7	*
4-1	4-s-Butoxy-2-butanone	5	
4-2	2,5-Dimethyl-4-methoxy-3(2H)-furanone	10	***
4-2	3-Pentanone	5	*
5	<b>All acids</b>	6	
5-1	(E)-3-Hexanoic acid	3	
5-2	Hexanoic acid	10	***
6	<b>All aromatics</b>	10	***
6-1	Veratrole	3	
6-2	Naphthalene	4	
6-3	Guaiacol	5	
6-4	Methyl eugenol	7	*

Note: <sup>a</sup> In the triangulation experiment, the number of evaluators who correctly identified the missing model. <sup>b</sup> significant: \*, significant ( $\alpha \leq 0.05$ ). \*\*, highly significant ( $\alpha \leq 0.01$ ); \*\*\*, very highly significant ( $\alpha \leq 0.001$ ), no \* indicates insignificant.

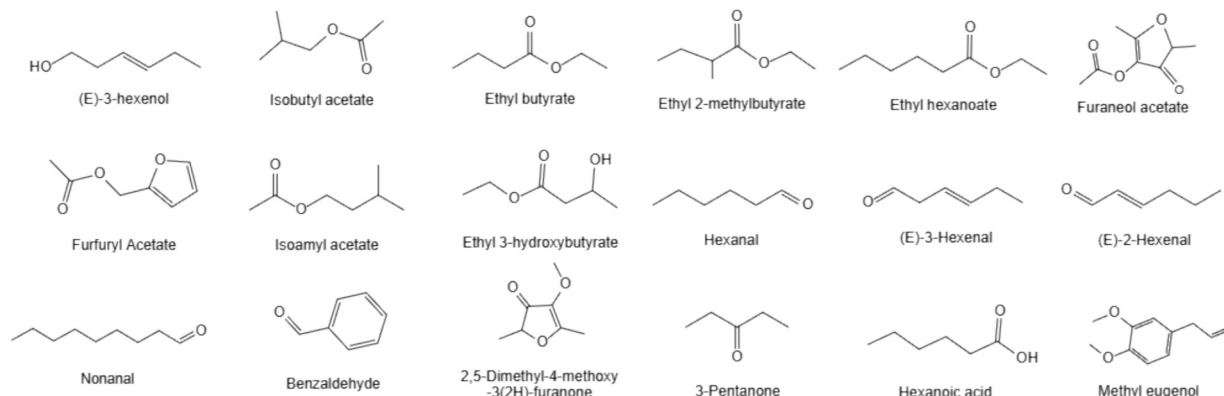
### 3.7. Interactions of key aroma compounds

Key aroma compounds of RRT further screened by the omission tests were mixed according to the quantitative concentrations and binary mixtures were established in pairs to explore the interactions between key aroma compounds. Given that the concentration of essential fragrance components in RRT might vary depending on the circumstances, the real concentration was taken as the intermediate point, and two binary mixtures of lower and higher concentration were set respectively with a two-fold relation, to explore whether the possible concentration fluctuations would affect the key aroma chemicals'

interactions in RRT. The results of the interaction relationship between key aroma substances in RRT are shown in Fig. 4, 1 (red) represents "complete addition", 2 (orange) represents "synergy", 3 (apricot) represents "partial addition", 4 (light blue) represents "compromise", and 5 (dark blue) represents "masking". A total of 153 groups of binary mixtures were established for 18 key aroma compounds, of which 15 groups of binary mixtures had synergistic effect, 94 groups of binary mixtures had partial addition effect, 1 group of binary mixtures had full addition effect, 41 groups of binary mixtures had compromise effect, and 2 groups of binary mixtures had masking effect. Binary mixtures where synergies exist include: Methyl eugenol + Hexanoic acid, Hexanoic acid+3-Pentanone, 2, 5-Dimethyl-4-methoxy-3(2H)-furanone+3-Pentanone, 3-Pentanone + Nonanal, 3-Pentanone + Furaneol acetate, 3-Pentanone + Furfuryl acetate, 3-Pentanone + Isoamyl acetate, 3-Pentanone + (E)-3-hexenal, 3-Pentanone + Ethyl butyrate, 3-Pentanone + Isobutyl acetate, 3-Pentanone + Ethyl hexanoate 3-Pentanone + Ethyl 3-hydroxybutyrate, 3-Pentanone + (E)-2-hexenal, 3-Pentanone + Ethyl 2-methylbutyrate. Binary mixtures with masking effects include (E)-3-hexenal + Furaneol acetate, Hexanal + Isobutyl acetate. The key aroma compounds in RRT showed partial addition and compromise effects.

Some of the signature volatile compounds of RRT underwent significant changes in their perceptible aromas both before and after blending. Ethyl butyrate, reminiscent of "fruity" prior to mixing, lost its characteristic scent upon combination with Furaneol acetate, yet when paired with Isoamyl acetate, it acquired a subtly distinctive wine-like nuance. An intriguing "grassy" note emerged from the blend of (E)-3-hexenol and Isoamyl acetate, while Ethyl hexanoate's fusion with Isoamyl acetate amplified its wine essence. Furfuryl acetate mixed with Isoamyl acetate gives a smell like "nail polish", which has previously been shown to be indeed composed of many esters (Ouyang et al., 2024). A baking aroma was produced by the combination of Furaneol acetate and Isoamyl acetate. Initially, the blend of Hexanoic acid and Isoamyl acetate revealed a faintly minty character, evolving into a banana-like aroma over time. The harmonious union of Methyl eugenol and Ethyl butyrate yielded a delightful aroma. While hexanal exudes a grassy quality on its own, this trait appears muted and diminishes upon blending with 3-pentanone. Before merging with 3-pentanone, isoamyl acetate bears a resemblance to rice wine; however, post-blend, the rice wine flavor dissipates. Ethyl butyrate, bearing a slight sourness before the addition of 3-pentanone, transforms into a robustly sweet scent. When Hexanoic acid and Ethyl hexanoate were combined, the sweetness diminished and the alcohol aroma intensified. The amalgamation of Hexanoic acid and Hexanal served to temper the "grassy" fragrance.

In conclusion, the interplay among the key aroma compounds within RRT precludes a simplistic superposition of individual scents. Instead, the additive interaction dynamics within RRT significantly influence its olfactory profile, demonstrating that the aroma of RRT is a complex result of synergistic interactions rather than a mere sum of its parts.



**Fig. 3.** Key aroma compounds in RRT.



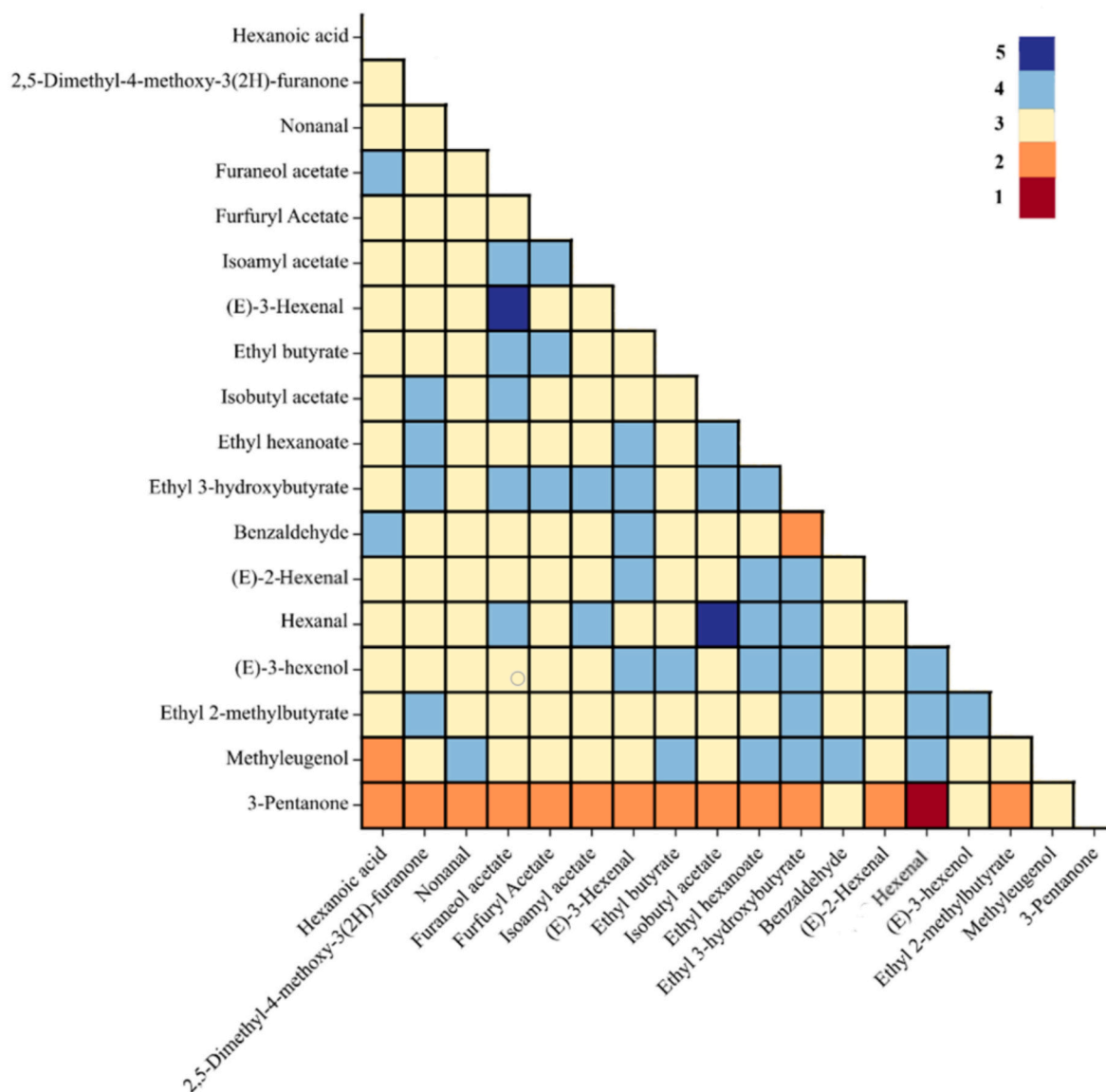


Fig. 4. Interaction between key aroma substances of RRT.

#### 4. Conclusions

This study employed sensomics to analyze the fragrance properties of RRT. A total of 99 volatile compounds, including acids, alcohols, ketones, esters, and aldehydes, were identified across different producing regions. The RRT aroma profile was characterized by grassy, caramel, floral, fruity, pear, tea-like, and woody notes. Gas chromatography-sniffing and the standard curve method were used to quantify 37 aroma-active compounds, revealing their contributions to RRT's aroma. Reconstitution of 29 aroma-active compounds with OAVs  $\geq 1$  in a simulated matrix closely matched the actual RRT aroma, except for an intensified grassy note.

Regional differences were also evident: LL samples had the highest volatile content, particularly in esters, terpenes, and aromatics, leading to a richer aroma. SC samples were notable for their high alcohol content but had lower levels of furans and aldehydes. DF samples exhibited the lowest overall volatile content, resulting in a more subdued aroma, while PZ samples had higher concentrations of acids, aldehydes, ketones, and furans, contributing to stronger grassy and woody notes but a less pronounced caramel scent. Omission studies identified 23

significant aroma models, with 6 highly significant, 6 very significant, and 11 significant. The final analysis highlighted 18 key aroma compounds crucial to RRT's characteristic fragrance, including 2,5-Dimethyl-4-methoxy-3(2H)-furanone, Isobutyl acetate, (E)-3-hexenol, and Hexanoic acid. These findings enhance the understanding of regional volatile profiles, offering insights for quality control and product standardization in RRT.

#### CRediT authorship contribution statement

**Xiaofang Sheng:** Writing – review & editing, Writing – original draft, Resources, Methodology, Formal analysis, Data curation. **Xinxin Lu:** Writing – review & editing, Methodology. **Weiyuan Tang:** Methodology, Funding acquisition. **Zhihai Yu:** Software. **Xiaozhu Liu:** Methodology. **Fei Zhang:** Methodology, Funding acquisition. **Qun Huang:** Writing – review & editing, Methodology. **Mingzheng Huang:** Writing – review & editing, Software, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2024.101892>.

## References

- Cameleyre, M., Lytra, G., Tempere, S., & Barbe, J.-C. (2015). *Journal of Agricultural and Food Chemistry*, 2015,63(44). <https://doi.org/10.1021/acs.jafc.5b03489>
- Dou, T. X., Shi, J. F., Li, Y., Bi, F. C., Gao, H. J., Hu, C. H., ... Dong, T. (2020). Influence of harvest season on volatile aroma constituents of two banana cultivars by electronic nose and HS-SPME coupled with GC-MS. *Scientia Horticulturae*, 265, Article 109214. <https://doi.org/10.1016/j.scienta.2020.109214>
- Fan, W. L., & Michael, C. Q. (2006). Characterization of aroma compounds of Chinese "Wuliangye" and "Jiannanchun" liquors by aroma extract dilution analysis. *Journal of Agricultural and Food Chemistry*, 54, 2695–2704. <https://doi.org/10.1080/10942912.2017.1309549>
- Feng, S., Huang, M. M., Crane, J. H., & Wang, Y. (2018). Characterization of key aroma active compounds in lychee (*Litchi chinensis* Sonn.). *Journal of Food and Drug Analysis*, 26(2), 497–503. <https://doi.org/10.1016/j.jfda.2017.07.013>
- Ge, Y. H., Li, X., Huang, M. Z., Huang, Z. X., Wu, M. M., Sun, B. Q., ... Li, N. (2023). Aroma correlation assisted volatile coupled network analysis strategy to unveil main aroma-active volatiles of *Rosa roxburghii*(2023). *Food Research International*, 169. <https://doi.org/10.1016/J.FOODRES.2023.112819>
- Grimm, J. E., & Steinhaus, M. (2019). Characterization of the major odor-active compounds in jackfruit pulp[J]. *Journal of Agricultural and Food Chemistry*, 67(20), 5838–5846. <https://doi.org/10.1021/acs.jafc.9b01445>
- Grosch, W. (1994). Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour and Fragrance Journal*, 9(4), 147–158.
- He, J. Y., Zhang, Y. H., Ma, N., Zhang, X. L., Liu, M. H., & Fu, W. M. (2016). Comparative analysis of multiple ingredients in *Rosa roxburghii* and *R. Sterilis* fruits and their antioxidant activities(2016). *Journal of Functional Foods*, 27, 29–41. <https://doi.org/10.1016/j.jff.2016.08.058>
- Huang, M., Li, T., Hardie, W. J., Tang, W., & Li, X. (2022). Comparative characterization and sensory significance of volatile compounds in *Rosa roxburghii* Tratt fruit from five geographic locations in Guizhou, China. *Flavour and Fragrance Journal*, 37(3), 163–180. <https://doi.org/10.1002/ffj.3694>
- Huang, Q., Dong, K., Wang, Q., Huang, X., Wang, G., An, F., & Luo, P. (2022). Changes in volatile flavor of yak meat during oxidation based on multi-omics. *Food Chemistry*, 371, Article 131103. <https://doi.org/10.1016/j.foodchem.2021.131103>
- Kim, M. K., Jang, H. W., & Lee, K. G. (2020). Characterization of key aroma-active compounds isolated from Omija fruit treated differently based on odor activity values and descriptive sensory analysis. *Foods*, 9(5), 638. <https://doi.org/10.3390/foods9050638>
- Li, Q. Y., Mo, H. R., Huang, M. Z., Li, T. T., Tang, W. Y., Li, X., ... Li, Y. J. (2022). Comparison of volatile components in *Rosa roxburghii* Tratt at different altitudes and picking periods. *Food and Fermentation Science & Technology*, 58(01), 97–106. <https://doi.org/10.3969/j.issn.1674-506X.2022.01-014>
- Li, T. T., Huang, M. Z., Tang, W. Y., Li, Q. Y., & Lou, K. N. (2021). Determination of volatile components in *Rosa roxburghii* Tratt juice and the analysis of its contribution for aroma. *Food and Fermentation Industries*, 47(04), 237–246. <https://doi.org/10.13995/j.cnki.11-1802/ts.025956>
- Li, X., Tu, Z., Sha, X., Li, Z., Li, J., & Huang, M. (2022). Effect of coating on flavor metabolism of fish under different storage temperatures. *Food Chemistry: X*, 100256, 13. <https://doi.org/10.1016/j.fochx.2022.100256>
- Liang, L. L., Han, L., Chen, X., & Shi, L. H. (1992). Study on volatile aroma components of RRT fresh fruit. *Chemical bulletin (05)*, 34-36+39. Doi:10.14159/j.cnki.0441-3776.1992.05.007.
- Lin, L., Wang, Y., Yang, J., Yang, X. S., & Zhang, X. (2020). Changes of flavor substances in *Rosa sterilis* before and after fermentation. *Chinese Brewing*, 39(06), 155–161. <https://doi.org/10.11882/j.issn.0254-5071.2020.06.030>
- Liu, J. X., Wang, J. F., Han, A. Q., & Sun, Y. X. (2016). Biosynthesis of Ester Compounds in Grape Wine &Relative Influencing Factors. *Liquor-Making Science & Technology*, 09, 43–47. <https://doi.org/10.13746/j.njkt.2016055>
- Lytra, G., Tempere, S., & Revel, G. D. (2012). Impact of perceptive interactions on red wine fruity aroma. *Journal of Agricultural and Food Chemistry*, 60(50), 12260–12269. <https://doi.org/10.1021/jf302918q>
- Ouyang, W. U., Liu, S. Q., Huang, Y. X., & Jiang, R. F. (2024). Detection of volatile organic compounds in nail polish products based on solid-phase microextraction. *Journal of Instrumental Analysis*, (09), 1450–1457.
- Roessler, E. B., Pangborn, R. M., Sidel, J. L., & Stone, H. (1978). Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests[J]. *Journal of Food Science*, 43(3), 940–943. <https://doi.org/10.1111/j.1365-2621.1978.tb02458.x>, 2010.
- Sheng, X. F., Huang, M. Z., Li, T. T., Li, X., Cen, S. Y., Li, Q. Y., ... Tang, W. Y. (2023). Characterization of aroma compounds in *Rosa roxburghii* Tratt using solvent-assisted flavor evaporation headspace-solid phase microextraction coupled with gas chromatography-mass spectrometry and gas chromatography-olfactometry. *Food Chemistry: X*, 2023, 18. <https://doi.org/10.1016/j.fochx.2023.100632>
- Staroscik, J. A., & Wilson, A. A. (1982). Seasonal and regional variation in the quantitative composition of cold-pressed lemon oil from California and Arizona[J]. *Journal of Agricultural and Food Chemistry*, 30(5), 835–837. <https://doi.org/10.1021/jf00113a009>
- Steinhaus, P., & Schieberle, P. (2007). Characterization of the key aroma compounds in soy sauce using approaches of sensomics [J]. *Journal of Agricultural and Food Chemistry*, 55(15), 6262–6269. <https://doi.org/10.1021/JF0709092>
- Vilanova, M., Zamuz, S., Silva, D. F. A., et al. (2011). Intraspecific Diversity of Yeast Associated to *Vitis vinifera* Albarino Must from Different Vineyard Ecosystems[J]. *Journal of the institute of brewing*, 117(2), 224–229. <https://doi.org/10.1007/s10681-008-9801-7>
- Xiao, Z. B., Zhu, J. C., Niu, Y. W., Wang, P. P., Sun, X. X., & Wang, R. L. (2018). Synergistic effect of aromatic compounds. *Journal of Food Science and Technology*, 36(04), 1–7. <https://doi.org/10.3969/j.issn.2095-6002.2018.04.001>
- Zhao, Z. D., Zhang, A. H., & Hong, F. (2007). Trends in the researches on the nutritional and medical value of *Rosa Roxburghii* Tratt and its products. *Journal of Environmental and Occupational*, 01, 82–84. <https://doi.org/10.3969/j.issn.1006-3617.2007.01.025>
- Zhou, Z., Ma, Q., Zhu, Y. C., Chen, C., & Pan, S. Y. (2015). Analysis of free and O-Glycosidically bound aroma active compounds of wild *Rosa roxburghii* juice by GC-O-MS. *Food Science*, 36(22), 80–84. <https://doi.org/10.7506/spkx1002-6630-201522014>